

ID NO:61, and most preferably, the crystal proteins which are encoded by the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:58, or SEQ ID NO:60, or a nucleic acid sequence which hybridizes to the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:58, or SEQ ID NO:60 under conditions of moderate to high stringency.

A second method for preparing a modified Cry1* crystal protein is a further embodiment of the invention. This method generally involves identifying a Cry1 crystal protein having one or more loop regions, introducing one or more mutations into one or more of the loop regions, and obtaining the resulting modified crystal protein. Preferred Cry1* crystal proteins preparable by either of these methods include the Cry1A*, Cry1B*, Cry1C*, Cry1D*, Cry1E*, Cry1F*, Cry1G*, Cry1H*, Cry1I*, Cry1J*, and Cry1K* crystal proteins, and more preferably, the Cry1Aa*, Cry1Ab*, Cry1Ac*, Cry1Ad*, Cry1Ae*, Cry1Ba*, Cry1Bb*, Cry1Bc*, Cry1Ca*, Cry1Cb*, Cry1Da*, Cry1Db*, Cry1Ea*, Cry1Eb*, Cry1Fa*, Cry1Fb*, Cry1Hb*, Cry1Ia*, Cry1Ib*, Cry1Ja*, and Cry1Jb* crystal proteins. Highly preferred proteins include Cry1Ca* crystal proteins, such as those comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59, or SEQ ID NO:61, and those encoded by a nucleic acid sequence having the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:58, or SEQ ID NO:60, or a nucleic acid sequence which hybridizes to the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:58, or SEQ ID NO:60 under conditions of moderate stringency.

Amino acid, peptide and protein sequences within the scope of the present invention include, and are not limited to the sequences set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59, and SEQ ID NO:61, and alterations in the amino acid sequences including alterations, deletions, mutations, and homologs. Compositions which comprise from about 0.5% to about 99% by weight of the crystal protein, or more preferably from about 5% to about

75%, or from about 25% to about 50% by weight of the crystal protein are provided herein. Such compositions may readily be prepared using techniques of protein production and purification well-known to those of skill, and the methods disclosed herein. Such a process for preparing a Cry1C* crystal protein generally involves the steps of culturing a host cell which expresses the Cry1C* protein (such as a *Bacillus thuringiensis* NRRL B-21590, NRRL B-21591, NRRL B-21638, NRRL B-21639, NRRL B-21640, NRRL B-21609, NRRL B-21610, or NRRL B-21592 cell) under conditions effective to produce the crystal protein, and then obtaining the crystal protein so produced. The protein may be present within intact cells, and as such, no subsequent protein isolation or purification steps may be required. Alternatively, the cells may be broken, sonicated, lysed, disrupted, or plasmolyzed to free the crystal protein(s) from the remaining cell debris. In such cases, one may desire to isolate, concentrate, or further purify the resulting crystals containing the proteins prior to use, such as, for example, in the formulation of insecticidal compositions. The composition may ultimately be purified to consist almost entirely of the pure protein, or alternatively, be purified or isolated to a degree such that the composition comprises the crystal protein(s) in an amount of from between about 0.5% and about 99% by weight, or in an amount of from between about 5% and about 90% by weight, or in an amount of from between about 25% and about 75% by weight, *etc.*

2.3 RECOMBINANT VECTORS EXPRESSING THE MUTAGENIZED *CRYI* GENES

One important embodiment of the invention is a recombinant vector which comprises a nucleic acid segment encoding one or more *B. thuringiensis* crystal proteins having a modified amino acid sequence in one or more loop regions of domain 1, or between α helix 7 of domain 1 and β strand 1 of domain 2. Such a vector may be transferred to and replicated in a prokaryotic or eukaryotic host, with bacterial cells being particularly preferred as prokaryotic hosts, and plant cells being particularly preferred as eukaryotic hosts.

The amino acid sequence modifications may include one or more modified loop regions between α helices 1 and 2, α helices 2 and 3, α helices 3 and 4, α helices 4 and 5,

α helices 5 and 6, or α helices 6 and 7 of domain 1, or between α helix 7 of domain 1 and β strand 1 of domain 2. Preferred recombinant vectors are those which contain one or more nucleic acid segments which encode modified Cry1A, Cry1B, Cry1C, Cry1D, Cry1E, Cry1F, Cry1G, Cry1H, Cry1I, Cry1J, or Cry1K crystal proteins. Particularly preferred recombinant vectors are those which contain one or more nucleic acid segments which encode modified Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ad, Cry1Ae, Cry1Ba, Cry1Bb, Cry1Bc, Cry1Ca, Cry1Cb, Cry1Da, Cry1Db, Cry1Ea, Cry1Eb, Cry1Fa, Cry1Fb, Cry1Hb, Cry1Ia, Cry1Ib, Cry1Ja, or Cry1Jb crystal proteins, with modified Cry1Ca crystal proteins being particularly preferred.

In preferred embodiments, the recombinant vector comprises a nucleic acid segment encoding the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59, or SEQ ID NO:61. Highly preferred nucleic acid segments are those which have the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:58, or SEQ ID NO:60.

Another important embodiment of the invention is a transformed host cell which expresses one or more of these recombinant vectors. The host cell may be either prokaryotic or eukaryotic, and particularly preferred host cells are those which express the nucleic acid segment(s) comprising the recombinant vector which encode one or more *B. thuringiensis* crystal protein comprising modified amino acid sequences in one or more loop regions of domain 1, or between α helix 7 of domain 1 and β strand 1 of domain 2. Bacterial cells are particularly preferred as prokaryotic hosts, and plant cells are particularly preferred as eukaryotic hosts

In an important embodiment, the invention discloses and claims a host cell wherein the modified amino acid sequences comprise one or more loop regions between α helices 1 and 2, α helices 2 and 3, α helices 3 and 4, α helices 4 and 5, α helices 5 and 6 or α helices 6 and 7 of domain 1, or between α helix 7 of domain 1 and β strand 1 of domain 2. A particularly preferred host cell is one that comprises the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59, or SEQ ID NO:61, and more preferably, one that